Pharmacology Biochemistry & Behavior, Vol 27, pp 451–456 © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Different Genes Specify Hyporesponsiveness to Seizures Induced by Caffeine and the Benzodiazepine Inverse Agonist, DMCM

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Received 12 January 1987

SEALE, T W , K A ABLA, T H RODERICK, O M RENNERT AND J M CARNEY *Different genes specify hyporesponsiveness to seizures induced by caffeine and the benzodiazepine inverse agonist, DMCM* PHARMACOL BIOCHEM BEHAV 27(3) 451–456, 1987 —Two strains of inbred mice differed significantly in their susceptibility to tonic seizures induced by caffeine and the benzodiazepine inverse agonist, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) The hyporesponsive strain, SWR, was not less susceptible to the convulsant action of other chemical convulsants, an observation which indicated that the response differences between the strains were pharmacologically specific These observations and drug interaction studies suggested that caffeine-induced seizures might be mediated through an "inverse" agonist-like action of caffeine on benzodiazepine receptors associated with GABA receptor-benzodiazepine receptor-chloride ionophore complex To determine whether the coincident alteration in susceptibility to DMCM and caffeine resulted from a single mutational change or was the result of two different genetic changes occurring coincidentally between these two strains of mice, progeny from conventional Mendelian crosses (F_1, F_2 and reciprocal backcrosses) were analyzed for the co-segregation of susceptibility to DMCM and caffeine The inheritance of DMCM sensitivity was consistent with a single autosomal gene determinant in which the allele specifying increased responsiveness was dominant to the allele determining hyporesponsiveness The frequent occurrence of recombinant phenotypes (e g , caffeine hyporesponsive but DMCM sensitive mice) among F_2 and backcross progeny established that different genetic determinants encode DMCM susceptibility and caffeine susceptibility in these two strains of mice Thus, while these data establish a simply inherited difference in benzodiazepine responsiveness between the two mouse strains, they also indicate that this pair of strains is inappropriate for a genetic analysis aimed at probing the relationship between caffeine-induced seizures and the benzodiazepine receptor

Caffeine Benzodiazepine inverse agonist β-Carbolines Tonic seizures Behavior genetics Inbred mice

INCLUDED among the behavioral actions of caffeine is the ability of high doses of this central nervous system (CNS) stimulant to induce seizures Although this phenomenon is not well understood at the neurochemical level, several lines of evidence have implicated the direct action of caffeine upon "central type" benzodiazepine receptors [8, 9, 11, 15, 20–22] The benzodiazepine receptor-γ-aminobutyric acid (GABA) receptor-chloride ionophore complex is known to exert a significant inhibitory control upon the activity of CNS neurons [1,6] The potent antiepileptic action of benzodiazepines is correlated with their ability to bind to "central type" benzodiazepine receptors associated with this complex [1] Benzodiazepine receptors appear to be unique in their response to effector ligands In addition to agonists (which induce direct behavioral effects upon binding to the benzodiazepine receptor) and antagonists (which are without a direct biological effect but compete with agonists for binding to these receptors), a third class of effector ligands, the inverse agonists, have been identified [1, 2, 4, 12] These compounds appear to induce the opposite neurochemical and behavioral effects elicited by binding of agonists to benzodiazepine receptors [2,10] Instead of exhibiting the antianxiety and anticonvulsant actions of benzodiazepine agonists, inverse agonists, such as methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), are anxiogenic and exhibit proconvulsant and convulsant actions [2, 10, 12,

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15] Caffeine may act at high doses as a benzodiazepine inverse agonist Methylxanthine stimulants such as caffeine and theophylline competitively inhibit [^{3}H] diazepam binding to brain benzodiazepine receptors *in vitro* [8,9] The most potent of the methylxanthines in displacing [^{3}H] diazepam is caffeine which has an IC_{50} value well within the range that is achievable *in vivo* [8,9] Since caffeine has a significantly lower potency for displacing ligands which bind to "peripheral type" benzodiazepine binding sites than it does for ligands which bind to "central type" sites, this interaction of caffeine with benzodiazepine binding sites appears to be receptor-specific [20] Benzodiazepine agonists inhibit caffeine-induced seizures with a rank order of potency that parallels their affinities for binding to "central type" sites *in vitro* Inosine, a purine which may be an endogenous benzodiazepine ligand, also antagonizes caffeine-induced seizures [9,21] Ro 15-1788, a "central type" benzodiazepine antagonist, blocks caffeine-induced seizures [22] These observations, taken together, have suggested that caffeine at high doses acts *in vivo* as a "central type" benzodiazepine inverse agonist

To better understand the genetic, pharmacological and neurochemical bases for variation in human behavioral responsiveness to methylxanthines [16] and other CNS stimulants, we have undertaken a systematic characterization of inherent variation in behavioral responsiveness to caffeine in a model mammalian system, the inbred mouse Variants with altered responsiveness to the behavioral actions of both low and high doses of caffeine and other methylxanthines have been identified previously [7, 14, 16] Both efficacy-limited and potency-limited alterations in behavioral responsiveness have been found [7, 14, 15] These differences in behavioral responsiveness to methylxanthine administration do not appear to result from alterations in the compartmentation or catabolism of caffeine [3, 13, 14] Changes in caffeine responsiveness between inbred mouse strains can be behavior-specific, and, if more than one behavioral response differs between a pair of strains, each can be controlled by a different gene or set of genes [16–18] Two inbred mouse strains, SWR and CBA, differ markedly in their susceptibility to caffeine-induced tonic seizures and death [15,16] This behavioral trait appears to be under control of a single Mendelian gene [17] Recently we proposed that this well characterized difference in response to the convulsant action of caffeine might provide a genetic approach to elucidating the neurochemical mechanism(s) underlying methylxanthine-induced seizures [15,17] Further support for the involvement of "central type" benzodiazepine receptors in caffeine-induced seizures was drawn from our observation that the difference in susceptibility to the convulsant action of caffeine between SWR and CBA mice was pharmacologically-specific and that coincident hyporesponsiveness to caffeine-, and the benzodiazepine inverse agonist, DMCM, -induced seizures occurred in the SWR strain [15] We now report the results of genetic analyses designed to determine whether a single gene mutation or changes in more than one gene encode the coincident alteration in susceptibility to caffeine- and DMCM-induced seizures which occurs in the SWR strain of inbred mice

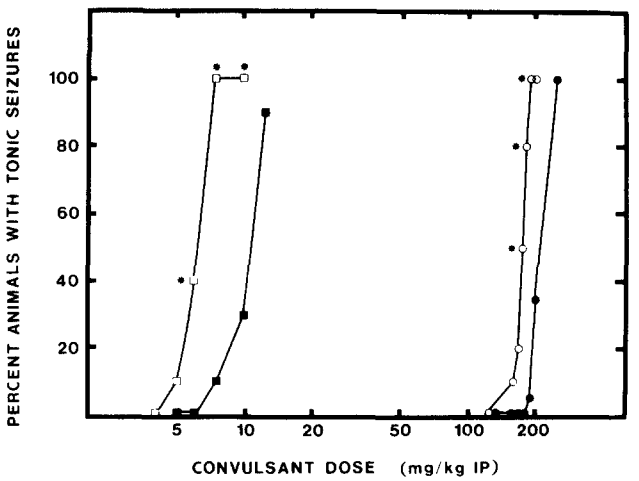

FIG 1 Susceptibility of SWR and CBA inbred mice to caffeine- and DMCM-induced tonic convulsions and death The dosage dependent induction of seizures occurs over a range of 5–15 mg/kg when DMCM is administered The SWR strain is significantly hyporesponsive to the induction of seizures by DMCM compared to the CBA strain Significantly higher doses of caffeine are required to induce seizures in both strains (>150 mg/kg) SWR also is hyporesponsive to the convulsant effect of caffeine Each point is the percentage of mice (n=10) seizing after administration of one of the two convulsants Caffeine [SWR (●), CBA (○)], DMCM [SWR (■), CBA (□)] *Indicates significant difference between the two strains

METHOD

Animals

Adult male mice of inbred strains CBA/J and SWR/J (Jackson Laboratory, Bar Harbor, ME) approximately 3 months of age were housed in groups of 5 animals per cage on a continuous 12 hour light-dark cycle under constant humidity and temperature (19–21°C) The litter used was hardwood chips (Sani-chips, P J Murphy) Free access to a standard pellet food (Lab/Blox, Wayne) and water were given Throughout this study we examined the effects of convulsants only on male animals Conventional genetic crosses were used because no recombinant inbred lines are available for this pair of inbred mouse strains Male F_1 hybrid progeny were obtained from reciprocal crosses of the two parental strains Male F_2 progeny were obtained from crosses of F_1 hybrid males and females Backcross progeny were obtained from crosses of F_1 hybrid females to parental males of each strain Unless otherwise specified, animals were drug naive and were used for only a single administration of drug

Convulsants

Caffeine (Sigma Chemical Co) solutions were freshly prepared in physiological saline containing 5 mM NaOH DMCM (Research Biochemicals) was dissolved in a 1 1 mixture by weight of dimethylsulfoxide (Fisher Scientific) and Emulphor (Emulphor EL-620, GAF Corp) and then was diluted with physiological saline to give a final vehicle composition of 30% dimethylsulfoxide-Emulphor to 70% saline DMCM solutions were prepared immediately before injection and administered in a volume of 0 1 ml/animal

Seizure Susceptibility Testing

Stress-potentiated death We previously developed a simple, quantitative method for stress potentiation of caffeine-induced tonic seizures and death [15] A swim stress, achieved by gently placing individual animals in a large beaker of water, gives reproducible results which can

TABLE 1

PREDICTED AND OBSERVED SEGREGATION PATTERNS FOR DMCM SUSCEPTIBILITY IN CROSSES DERIVED FROM CBA AND SWR INBRED MICE

Origin of Strains or Progeny	Expected Genotype(s)	Expected Phenotype	Observed Phenotype Percentage Survivors
Parental Strains			
CBA	dmc^s/dmc^s	sensitive	0% (0/20)
SWR	dmc^r/dmc^r	resistant	100% (29/30)
F_1 hybrids			
CBA × SWR	dmc^s/dmc^r	all sensitive or	0% (0/10)
SWR × CBA	dmc^s/dmc^r	all resistant	0% (0/10)
Backcrosses			
F_1 × SWR	1 dmc^s/dmc^r	50% sensitive	56% (28/50)
	1 dmc^r/dmc^r	50% resistant	
F_1 × CBA	1 dmc^s/dmc^r	100% sensitive	0% (20/20)
	1 dmc^s/dmc^s		
F_2 (selfed F_1 cross)			
$CBSWF_1 \times CBSWF_1$	1 dmc^s/dmc^s	75% sensitive	32% (23/72)
	2 dmc^s/dmc^r	25% resistant	
	1 dmc^r/dmc^r		

The numbers in parentheses are the observed number of surviving animals divided by the total number of animals tested Screening for sensitivity to DMCM-induced tonic convulsions was carried out at a dose of 7 5 mg/kg IP *dmc* designates the gene encoding relative susceptibility to DMCM, with 2 alleles, *(r)* resistant, and *(s)* susceptible

be quantitated in terms of time of onset and frequency of tonic seizures and death Twenty minutes after caffeine administration, mice were subjected to a swimming stress by placing individual animals in a 2 liter beaker containing water at 25°C Untreated or non-responding animals swim actively for >2 min and do not have tonic seizures or die Inactive animals float Induction of tonic seizures and death occur in <2 min of responding strains such as CBA Animals appear to die of respiratory arrest following tonic seizures, not drowning An individual test is scored as positive when seizures and death occurs in <2 min

Tonic seizures inducted by DMCM Tonic seizures and death, scored according to the behavioral description of Seyfried [19], were determined for 30 minutes following intraperitoneal administration of DMCM Because of the diurnal and temperature effects known to modulate convulsant sensitivity, experiments were conducted between 0900 and 1600 hours at 19–21°C Dosing and strains were staggered so as to avoid complications brought about by strain differences in diurnal rhythm The occurrence of a tonic seizure was scored as positive when all four legs of an animal were rapidly extended to the rear Respiratory arrest and death usually but not always followed the occurrence of a tonic seizure All comparisons of dose dependent responses between the SWR or CBA strains or their F_1 derivatives were made directly by simultaneously injecting both strains with the same drug solution

Statistical Testing

Comparison of the behavioral responses of individual mouse strains to various doses of the convulsants was made by the Fisher Exact Method [5] The Chi-square method was used to compare the frequencies of behavioral classes observed in backcross and F_2 progeny to those expected for various hypothetical models A value of $p<0$ 05 was taken as statistically significant

RESULTS AND DISCUSSION

Susceptibility of SWR and CBA Inbred Mice to Lethal Seizures Induced by Caffeine and DMCM

The relative susceptibilities of SWR and CBA inbred mice to tonic seizure induction by caffeine and DMCM are shown respectively in Fig 1 Stress-potentiated seizures were used to assess the relative responsiveness of the two strains of mice to caffeine because we found that this behavioral assay more reproducibly identifies significant differences in response to caffeine between these strains [16] CBA mice are significantly more responsive to caffeine-induced tonic seizures at doses 170 mg/kg IP A dose of 187 mg/kg IP induced 100% tonic seizures and death in CBA but less than 5% of SWR mice tonically seize and die at this dose ($p<0$ 001) This dose of caffeine was chosen as the screening dose for the cross analyses to investigate the genetic determinants of susceptibility to caffeine and DMCM

We found that CBA mice also are more susceptible to the convulant action of DMCM than are SWR mice Previously we showed that this difference in convulsant sensitivity between the two strains of mice was pharmacologically specific [15] Other convulsants, such as picrotoxinin or

strychnine, were equally potent in their ability to induce tonic seizures in the two strains of mice The CD_{50} dose for DMCM-induced tonic seizures is about 2-fold higher in SWR mice than in CBA mice Doses of 6 mg/kg IP readily distinguish the seizure susceptibility of the two strains We chose a DMCM dose of 7 5 mg/kg IP which maximizes the difference in responsiveness between the two strains ($p<0$ 001) as the screening dose to be used in the genetic analyses of cross progeny

Evidence That the Difference in Susceptibility to the Convulsant Action of DMCM Between SWR and CBA Mice Is a Single Gene Effect

To determine whether the relative susceptibility to DMCM-induced seizures was controlled in a simple Mendelian manner, we characterized the convulsant response of 162 male progeny mice from five different conventional genetic crosses involving the SWR strain, the CBA strain and their hybrid derivatives These data are summarized in Table 1 The simplest testable hypothesis to explain the inherent alteration in DMCM sensitivity is that a pair of homozygous alleles differs at a single gene locus between the SWR and CBA inbred strains This hypothesis is illustrated in Table 1 Sensitivity to DMCM in the CBA strain is symbolized by homozygosis of the sensitive allele, *s*, at a genetic locus (*dmc*) determining susceptibility to this convulsant Similarly, resistance to DMCM-induced convulsions is symbolized by the *r* allele which is homozygous at the *dmc* locus in the SWR strain In such an hypothesis, F_1 hybrid male progeny from reciprocal crosses are expected to be heterozygous if the *dmc* gene is autosomal The phenotype of these F_1 progeny would depend upon the dominance relationship of the *r* and *s* alleles Our results indicate that all male progeny from both reciprocal crosses were sensitive to DMCM-induced tonic seizures, i e , susceptibility to DMCM, rather than resistance, is dominant The *dmc* gene(s) appears to be located on an autosome rather than on a sex chromosome Males derived from reciprocal F_1 crosses ought to resemble their mothers in phenotype (i e , the male progeny from the two crosses would have different sensitivities to DMCM-induced convulsions) if the *dmc* gene were located on their maternally-derived X chromosome Since no differences in DMCM susceptibility between progeny of the two F_1 crosses were noted, the genetic determinants for the difference in convulsant responsiveness must reside on an autosome

When F_1 progeny animals were backcrossed to each of their parents, two different results were observed (Table 1) If SWR mice are considered to be homozygous resistant (dmc^r/dmc^r), two genotypes are expected among the progeny of the cross of F_1 X SWR mice—dmc^s/dmc^r and dmc^r/dmc^r Since these genotypes are expected to occur with equal frequencies, the phenotypic ratio among the progeny of this cross should be one DMCM sensitive to one DMCM resistant In contrast, when F_1 mice are crossed to their CBA parent, all progeny are expected to carry a dmc^s allele, and, thus, to be relatively more susceptible to DMCM-induced seizures (Table 1) When 70 male mice from these reciprocal backcrosses were analyzed, their phenotypic ratios closely approximated these expectations Both DMCM resistant and DMCM sensitive progeny were observed in the backcross of F_1 mice to SWR Twenty-eight of 50 progeny mice from this cross (56%) were resistant to DMCM-induced tonic seizures This frequency of DMCM resistant progeny is not significantly different from the expected frequency based upon the segregation of a single pair of alleles ($\chi^2=0$ 72, $p>0$ 3) No such DMCM resistant mice (0 of 20) were found among the progeny of the backcross of F_1 mice to the CBA strain Results from both of these backcrosses suggest a single gene difference accounts for the difference in DMCM susceptibility between the CBA and SWR strains of mice

Another way to test whether relative susceptibility to DMCM-induced tonic seizures is due to the difference in a single pair of alleles is by examining the progeny which result from self-crossing of F_1 male progeny to F_1 female progeny Table 1 identifies the expected genotypes and phenotypes based upon a single gene model for determination of DMCM sensitivity Genotypically, one-fourth of the progeny are expected to be homozygous sensitive, one-half heterozygous for sensitivity and one-fourth homozygous resistant We found 23 of 72 F_2 progeny mice (32%) were resistant This ratio is not significantly different from the predicted ratio ($\chi^2=1$ 85, $p=0$ 2) Since the phenotype of the F_1 progeny established that heterozygotes are sensitive to DMCM, the ratio of phenotypes among the F_2 progeny is expected to be 3 DMCM sensitive to 1 DMCM resistant These data further support the hypothesis that the difference in relative susceptibility to DMCM-induced seizures between CBA and SWR inbred mice is determined by a single pair of alleles

Evidence That Different Genes Encode Relative Susceptibility to DMCM- and Caffeine-Induced Tonic Seizures in SWR and CBA Mice

Previously we have shown that the inherent alteration in susceptibility to the convulsant effects of caffeine in the SWR and CBA strains is due to a single gene difference between them [17] Because of the data from other laboratories and ours [8, 9, 11, 15, 20–22] implicating brain "central type" benzodiazepine receptors in the convulsant action of caffeine, we conjectured that the coincident hyporesponsiveness of the SWR strain to both caffeine and DMCM might arise from a single mutational event which alters the function or number of these receptors [15] In this model, high dosages of caffeine were envisioned to act like a benzodiazepine inverse agonist with properties similar to those of DMCM A genetic test of this single gene hypothesis is to determine if susceptibilities to caffeine- and DMCM-induced seizures co-segregate in progeny from genetic crosses If a single gene alteration causes the coincident change in susceptibilities to both caffeine and DMCM, only parental phenotypic responses to the two convulsants (hyporesponsiveness to both or sensitivity to both) are expected among cross progeny, i e , responsiveness to the two convulsants co-segregates (Table 2) However, if altered susceptibilities to the two convulsants are due to mutations in two separate genes, then in addition to the parental phenotypic classes, genetic recombination should produce new, non-parental recombinant genotypes and phentoypes in cross progeny (i e , mice which are hyporesponsive to one of the convulsants but resistant to the other) (Table 2)

Two genetic crosses provide a test of these alternative hypotheses—the backcross of the F_1 hybrid to SWR and the selfed cross of the F_1 hybrids to produce the F_2 generation Because sensitivity to both convulsants is dominant to resistance, the backcross of F_1 mice to their CBA progenitor will not be informative A way to invalidate the co-segregation hypothesis is to identify caffeine sensitive progeny

TABLE 2

PREDICTED AND OBSERVED ASSORTMENT PATTERNS FOR SUSCEPTIBILITY TO DMCM AND CAFFEINE IN CROSSES DERIVED FROM CBA AND SWR INBRED MICE

Characteristic	Single Gene Model	Two Gene Model	Observed Phenotype Percentage Survivors of Caffeine Treatment Among DMCM Insensitive Progeny
Cross of Parents	$F_1 \times$ SWR	$F_1 \times$ SWR	
Genotype of Parents	$dmc^s/dmc^r \times dmc^r/dmc^r$	$dmc^s/dmc^r,caf^s/caf^r \times dmc^r/dmc^r,caf^r/caf^r$	
Progeny Genotypes	1 dmc^s/dmc^r 1 dmc^r/dmc^r	1 $dmc^s/dmc^r,caf^s/caf^r$ 1 $dmc^r/dmc^r,caf^s/caf^r$ 1 $dmc^s/dmc^r,caf^r/caf^r$ 1 $dmc^r/dmc^r,caf^r/caf^r$	
Progeny Phenotypes	1 resistant to both caffeine and DMCM 1 sensitive to both caffeine and DMCM	1 sensitive to both caffeine and DMCM 1 resistant to DMCM, sensitive to caffeine 1 sensitive to DMCM, resistant to caffeine 1 resistant to both DMCM and caffeine	86% (25/29)
Cross of Parents	$F_1 \times F_1$	$F_1 \times F_1$	
Genotype of Parents	$dmc^s/dmc^r \times dmc^s/dmc^r$	$dmc^s/dmc^r,caf^s/caf^r \times dmc^s/dmc^r,caf^s/caf^r$	
Progeny Genotypes	1 dmc^s/dmc^s 2 dmc^s/dmc^r 1 dmc^r/dmc^r	1 $dmc^s/dmc^s,caf^s/caf^s$ 2 $dmc^s/dmc^s,caf^s/caf^r$ 1 $dmc^s/dmc^s,caf^r/caf^r$ 2 $dmc^s/dmc^r,caf^s/caf^s$ 4 $dmc^s/dmc^r,caf^s/caf^r$ 2 $dmc^s/dmc^s,caf^r/caf^r$ 1 $dmc^r/dmc^r,caf^s/caf^s$ 2 $dmc^r/dmc^s,caf^s/caf^r$ 1 $dmc^r/dmc^r,caf^r/caf^r$	
Progeny Phenotypes	3 sensitive to both DMCM and caffeine 1 resistant to both DMCM and caffeine	9 sensitive to both DMCM and caffeine 3 sensitive to DMCM, resistant to caffeine 3 resistant to DMCM, sensitive to caffeine 1 resistant to both DMCM and caffeine	60% (26/43)

The numbers in parentheses are the observed number of surviving animals divided by the total number of animals tested Screening for sensitivity to caffeine-induced seizures (187 mg/kg IP) and death among survivors of DMCM administration In the single gene model, the susceptibility allele, *s*, determines sensitivity to either caffeine or DMCM In the two gene model, one gene, *dmc*, specifies susceptibility to DMCM, and a second gene, *caf* specifies susceptibility to caffeine

among those progeny mice which failed to have tonic seizures following DMCM administration Limitation of the supply of progeny from these crosses precluded the assessment of the frequency of occurrence of the reciprocal recombinant class, DMCM sensitivity in caffeine resistant mice When DMCM resistant progeny from the cross of F_1 hybrids to their SWR parent were examined for their susceptibility to caffeine-induced seizures, animals sensitive to caffeine were frequently observed Of 29 DMCM resistant progeny examined from this cross, 4 (14%) were found to be caffeine sensitive These recombinants were not mistakenly classified because a subgroup of these DMCM insensitive mice were retested for DMCM susceptibility and found to be resistant to a second DMCM challenge before caffeine administration Similarly, ten mice were retested for caffeine sensitivity and, on the second test, all still showed caffeine resistance Among 43 DMCM resistant mice tested from the F_2 generation, 17 (40%) were sensitive to caffeine-induced convulsions and death (Table 2) The identification of a significant number of animals with recombinant phenotypes among the progeny of these two crosses clearly establishes that different genes respectively encode susceptibility to caffeine and DMCM However, the frequency of occurrence of the recombinant classes that we observed differs significantly from the value expected for random assortment of the two genes For example, in progeny of the F_2 generation, three-fourths of the DMCM resistant animals are expected to be caffeine sensitive and one-fourth of the mice are expected to be caffeine resistant We found that only 40% (17 of 43) of these progeny mice were caffeine sensitive, a significant departure from the expected ratio ($\chi^2=27\ 48$, $p<<0\ 001$) This excess in the frequency of a parental phenotypic class suggests that the two individual genes determining caffeine and DMCM susceptibility do not assort independently but are genetically linked on the same chromosome Analysis of a much larger number of animals than was available for the current study is necessary to confirm this linkage relationship and to determine the map distance between the two genes

The unequivocal experimental support for the involve-

ment of two different genes encoding differences in susceptibility to caffeine and DMCM establishes that the hyposensitivity of SWR mice to these two convulsants is genetically coincidental, not a coincident phenotypic change resulting from a single mutational event This *in vivo* finding does not necessarily obviate the concept that caffeine-induced seizures are mediated through a direct action on the benzodiazepine receptor [8, 9, 20–22] The data do establish that the coincident, pharmacologically-specific difference in convulsant responsiveness in this pair of inbred mouse strains does not provide a direct genetic test of the hypothesis However, our genetic analysis provides evidence for the existence of genetically controlled components of susceptibility to these two convulsants which can be mutationally altered in a specific manner Intrinsic susceptibility to tonic seizures induced by caffeine can be modified without significantly affecting susceptibility to tonic seizures induced by a benzodiazepine inverse agonist (and *vice versa*)

The present data describe the first single gene mutation which alters responsiveness to a benzodiazepine inverse agonist Although variation in behavioral responsiveness to benzodiazepines can occur because of altered metabolism or biodistribution [12], preliminary experiments (Seale, unpublished results) suggest that such a mechanism does not underlie the altered responsiveness to DMCM found between CBA and SWR mice Central nervous system-specific mutations leading to alterations in benzodiazepine receptor number, function and coupling to the GABA receptor-chloride ionophore complex hold considerable interest for the analysis of the role of this receptor and its endogenous ligand(s) in the intrinsic control of behavior and behavioral responsiveness to exogenously administered pharmacological agents

ACKNOWLEDGEMENTS

These studies were supported in part by a Research Scholar Award from the College of Medicine Alumni Association, University of Oklahoma Health Sciences Center to T W S , by a research contract from the International Life Science Institutes to J M C and by grants from the National Institute of Drug Abuse (DA 04028) and the Presbyterian Health Foundation The excellent technical assistance of Steve Langley with management of the genetic crosses is gratefully acknowledged The authors wish to express their appreciation to Holly Whiteside for her preparation of the manuscript

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